Subscriber access provided by ISTANBUL TEKNIK UNIV

Selective Hydrogenation of the C-C Double Bond of #,#-Unsaturated Carbonyl Compounds by the Immobilized Cells of Nicotiana tabacum

Takayuki Suga, and Yi-Xinog Tang

J. Nat. Prod., **1993**, 56 (8), 1406-1409• DOI: 10.1021/np50098a025 • Publication Date (Web): 01 July 2004

Downloaded from http://pubs.acs.org on April 4, 2009

More About This Article

The permalink http://dx.doi.org/10.1021/np50098a025 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

ACS Publications
High quality. High impact.

Journal of Natural Products is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Journal of Natural Products
Vol. 56, No. 8, pp. 1406-1409, August 1993

SELECTIVE HYDROGENATION OF THE C-C DOUBLE BOND OF α,β-UNSATURATED CARBONYL COMPOUNDS BY THE IMMOBILIZED CELLS OF *NICOTIANA TABACUM*[1]

TAKAYUKI SUGA* and YI-XINOG TANG

Department of Chemistry, Faculty of Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima, Hiroshima 724, Japan

ABSTRACT.—Immobilized cells of *Nicotiana tabacum* were found to convert more efficiently two α,β-unsaturated carbonyl compounds, (4*R*)-(−)-carvone [**1**] and (1*R*)-(+)-pulegone [**2**], to their corresponding saturated ketones and alcohols than the freely suspended cells. In addition, the formation of by-products, such as reduction products of the carbonyl group, could be depressed by carrying out the biotransformation in culture medium with pH near to the optimal pH of the enone reductases responsible for the reduction of the carbon-carbon double bond adjacent to the carbonyl group.

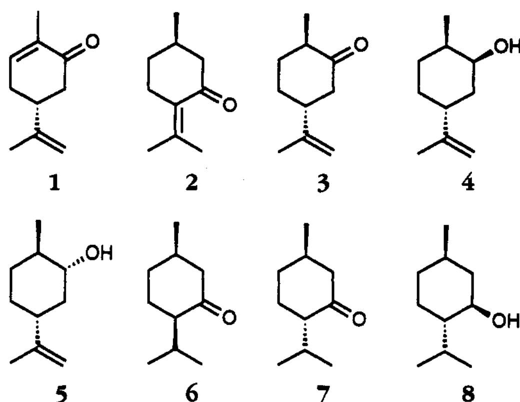
The biochemical potential of plant cell cultures to produce useful substances has been generally recognized, but the formation and accumulation of some secondary metabolites, especially monoterpenoids, do not normally occur in callus or cell suspensions of many plants (1,2).

However, there is evidence that such plant cell cultures retain abilities to convert exogenous substrates (3,4). Therefore, plant cell cultures are considered to be useful biocatalyst for reactions, such as hydroxylation at allylic positions, oxidoreduction between alcohols and ketones, reduction of carbon-carbon double bond, and hydrolysis (4). However, disadvantages such as the low efficiency of biotransformation and the formation of by-products always limit the direct application of the freely suspended cells. As shown in the previous papers (5,6), the freely suspended cells of *Nicotiana tabacum* L. (Solanaceae) could convert α,β-unsaturated carbonyl compounds, such as (4*R*)-(−)-carvone [**1**] and (1*R*)-(+)-pulegone [**2**], to the corresponding saturated ketones and alcohols. In the present work, we investigated the biotransformation of **1** and **2** by the immobilized cells of *N. tabacum* and found that the biotransformation efficiency was much higher than when using the freely suspended cells.

RESULTS AND DISCUSSION

Following the literature method (7), the imobilized cells of *N. tabacum* cell cultures were prepared by entrapping the suspension cells in calcium alginate gels. The immobilized cells were incubated with (4*R*)-(−)-carvone [**1**] and (1*R*)-(+)-pulegone [**2**] for a week. After the incubation, the cell beads were separated from the culture medium and homogenized. The homogenized cell beads and the medium were extracted separately with Et_2O. The total Et_2O extracts amounted to 94 and 93% of the administered **1** and **2**, respectively. Incubation of (4*R*)-(−)-carvone [**1**] with the immobilized cells gave (1*R*,2*S*,4*R*)-(+)-neodihydrocarveol [**4**] (85.4%) as the major product with minor amounts of (1*R*,4*R*)-(+)-dihydrocarvone [**3**] (1.9%) and (1*R*,2*R*,4*R*)-(−)-dihydrocarveol [**5**] (12.3%). Incubation of (1*R*)-(+)-pulegone [**2**] with the immobilized cells afforded (1*R*,4*R*)-(+)-isomenthone [**6**] (96.3%) as the major product with minor amounts of (1*R*,4*S*)-(−)-menthone [**7**] (1.2%) and (1*R*,3*R*,4*S*)-(+)-menthol [**8**] (0.9%). The products were identified by direct comparisons of the glc and glc-ms with those of the authentic samples. The time-courses in the biotransformations of **1** and **2** with the immobilized cells are

[1]Dedicated to the memory of the late Professor Edward Leete.



depicted in Figures 1a and 2a, respectively. For comparison, the time-courses in the biotransformations by the freely suspended cells were reproduced in Figures 1c and 2c, respectively, from our previous reports (5,6). The weight ratio of the cells to the substrates was 1500 in the biotransformation by the immobilized cells and 4000 by the freely suspended cells. The average conversion rate of **1** by the immobilized cells for 3 days was 0.213 mg/g fresh wt of the cells per day; some 7 times faster than by the freely suspended cells (0.03 mg/g fresh wt per day). The conversion rate of **2** was 0.218 mg/g fresh wt per day; some 5 times faster than by the freely suspended cells (0.05 mg/g fresh wt per day).

As has been reported previously (8), the reduction of the carbon-carbon double bonds of the (s)-trans and (s)-cis enone groups of **1** and **2** are catalyzed by two different enone reductases, "carvone reductase" and "pulegone reductase," forming **3** and **6** as the sole products, respectively. The two reductases showed different property and substrate specificity. The pH optima of the "carvone reductase" and "pulegone reductase" were 7.7 and 6.5, respectively. On the other hand, the alcohol-dehydrogenase-catalyzed reduction of the carbonyl group of the saturated ketones **3** and **6** is reported to show maximal activity at the optimal pH 6.8 (9). The higher conversion efficiency

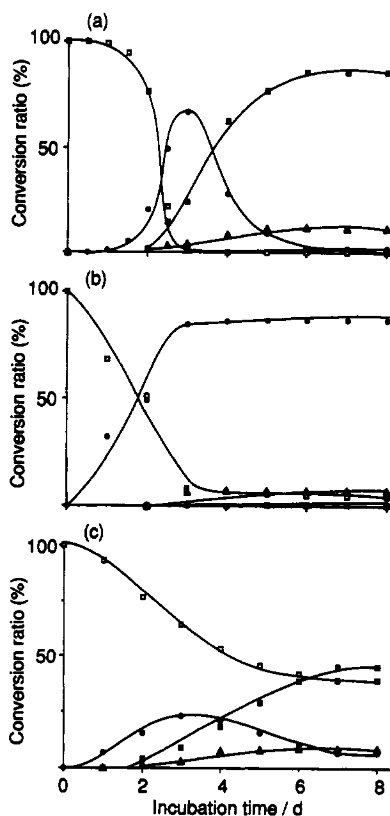


FIGURE 1. Time-courses in the biotransformation of (4*R*)-(−)-carvone [**1**] with the immobilized cells of *Nicotiana tabacum* (a) at pH 5.2 and (b) at pH 7.2, and (c) with the freely suspended cells at pH 5.2: -□- (4*R*)-(−)-carvone [**1**]; -●- (1*R*,4*R*)-(+)-dihydrocarvone [**3**]; -■- (1*R*,2*S*,4*R*)-(+)-neodihydrocarveol [**4**]; -Δ- (1*R*,2*R*,4*R*)-(+)-dihydrocarveol [**5**].

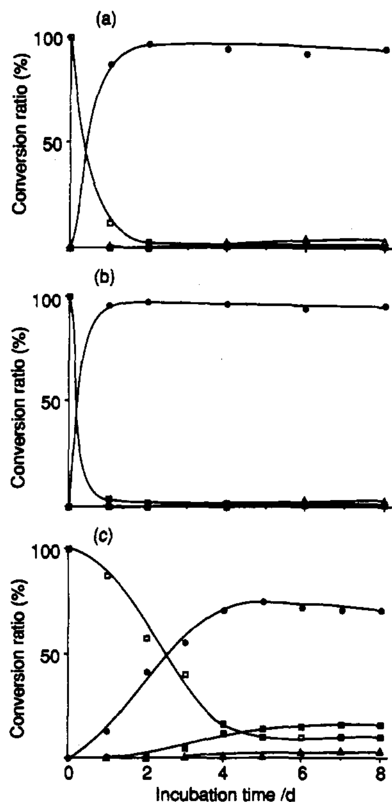


FIGURE 2. Time-courses in the biotransformation of (1*R*)-(+)-pulegone [**2**] with the immobilized cells of *Nicotiana tabacum* at (a) pH 5.2 and (b) pH 6.1, and (c) with the freely suspended cells at pH 5.2: -□- (1*R*)-(+)-pulegone [**2**]; -●- (1*R*,4*R*)-(+)-isomenthone [**6**]; -■- (1*R*,4*S*)-(−)-menthone [**7**]; -Δ- (1*R*,3*R*,4*S*)-(+)-menthol [**8**].

and the selective reduction of the carbon-carbon double bond could be expected by performing the reactions at pHs near the pH optima of the reductases. The freely suspended cells were susceptible to the pH changes of the medium, and no detectable biotransformation activity was retained over pH 6.7. However, no significant decrease of the activity in the biotransformation of **1** by the immobilized cells was observed up to pH 7.2. Figures 1b and 2b show the time-courses in the biotransformation of **1** at pH 7.2 and that of **2** at pH 6.1 with the immobilized cells, respectively. As shown in Figure 1b, the amount of **3** reached maximum (86.5%), and no further reduction of the carbonyl group of **3** was observed for prolonged incubation period, although the prolonged incubation resulted in the reduction of the carbonyl group at pH 5.2 (Figure 1a). Only small amounts of **4** (0.6%) and **5** (7.2%) were formed in 7 days. On the other hand, the biotransformation of **2** with the immobilized cells at pH 6.1 gave maximal **6** (97.4%) in 1 day (Figure 2b). As shown in Figures 2a and 2b, the similarity in the time-courses was observed between the biotransformations at pH 6.1 and 5.2, but the biotransformation at pH 6.1 was faster than that at pH 5.2. Our previous results (8) have demonstrated that the "pulegone reductase"-catalyzed hydrogen addition to the 4 position of **2** occurs stereospecifically from *si* face to give **6** exclusively. The formation of (1*R*,4*S*)-(−)-menthone [**7**] was probably due to another enone reductase or a menthone isomerase (10). No formation of the alcohol corresponding to **6** may be caused by the steric hindrance owing to the axially oriented 2-methylethyl group adjacent to the carbonyl group, because the alcohol dehydrogenase reduces the carbonyl group from *re* face stereospecifically (9).

Thus, we found that the immobilized cells of *N. tabacum* converted α,β-unsaturated carbonyl compounds to the corresponding saturated ketones and alcohols much faster than the freely suspended cells. It was also found that the product distribution in the biotransformation of the enone compounds by the immobilized cells depends greatly on the pH of the medium used; this provides us a convenient control to the biotransformation by cultured cells. The selective reduction of the carbon-carbon double bond adjacent to the carbonyl group could be enhanced by carrying out the biotransformation at pH near to the optimal pH of the enone reductase responsible for the reduction of the carbon-carbon double bond.

EXPERIMENTAL

The substrates used in this work, (4*R*)-(−)-carvone [**1**] and (1*R*)-(+)-pulegone [**2**], were the same as those used previously (8). Analytical and preparative tlc, glc, gc-ms, and ^{1}H-nmr measurements were made similarly to those as described previously (8).

PREPARATION OF THE IMMOBILIZED CELLS OF *N. TABACUM*.—Suspension cells of *N. tabacum* Bright Yellow subcultured for 3–4 weeks were used in the present work. Following the reported procedure (7), fresh cells (15 g) of *N. tabacum* were suspended in 2% sodium alginate (15 ml) and then poured dropwise into 0.1 M $CaCl_2$ solution (500 ml). The Ca-alginate beads with 3.5 mm mean diameter were in turn washed with sterilized H_2O (500 ml) and Murashige and Skoog's medium (11) containing 2 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 3% of sucrose (MS-D medium, 500 ml).

BIOTRANSFORMATION OF **1** AND **2** WITH THE IMMOBILIZED CELLS IN MEDIUM WITH DIFFERENT PH.—MS-D media were adjusted to pH 5.8, 6.5, and 7.5 with 0.1 M NaOH solution. After autoclaving at 121° for 20 min, the pHs of the media changed to 5.2, 6.1 and 7.2, respectively. No further pH adjustment of the autoclaved medium was made. The immobilized cells were suspended in 100 ml of the medium thus prepared. To the suspended immobilized cells, **1** or **2** (10 mg) was administered and then incubated at 25° for a week in the same manner as previously described (5,6). After the incubation, the cell beads were separated from the culture medium by filtration. The medium was extracted with Et_2O (3×200 ml). The cell beads were homogenized and extracted with Et_2O (3×200 ml). After removal of the solvent, 7.92 and 1.50 mg of biotransformation products of **1** were obtained from the culture medium and cell beads extracts, respectively. In the case of **2**, the amounts of the products extracted from the medium and cell beads were 7.80 and 1.50 mg, respectively. The Et_2O extracts from the medium and cell beads were combined, because they exhibited identical tlc behaviors. After purification of the combined extracts by preparative tlc, **3** (0.15 mg), **4** (8.20 mg), and **5** (1.05 mg) were obtained as the biotransformation products of **1**. In the case of **2**, **6** (9.13 mg), **7** (0.09 mg), and **8** (0.05 mg) were obtained. Time-course experiments were performed in the manner similar to that described previously for the freely suspended cells (5,6). Average conversion rates of the substrates for 3 days were calculated on the basis of the weight percentages of the unchanged substrates to the substrates administered.

IDENTIFICATION OF THE PRODUCTS.—The products yielded from **1** and **2** by incubation with the freely suspended cells were already identified exactly by the physical (n^{25}D and $[\alpha]^{25}$D) and spectral data (nmr, ms, ir, and uv) (5,6). In the present work, therefore, the products were identified by direct comparisons of the glc and gc-ms with those of the authentic samples.

ACKNOWLEDGMENTS

The authors thank Takasago Perfumery Co., Ltd. for a gift of the sample of (4*R*)-(−)-carvone. The present work was in part supported by Grant-in-Aids for Special Project Research No. 02250227 (1990, to T.S.), No. 03236230 (1991, to T.S.), and No. 04220228 (1992, to T.S.) and Cooperative Research No. 03303003 (1991) from the Ministry of Education, Science and Culture, Japan.

LITERATURE CITED

1. E.J. Staba, S. Zito, and M. Amin, *J. Nat. Prod.*, **45**, 256 (1982).
2. D.V. Banthorpe and S.E. Barrow, *Phytochemistry*, **22**, 2727 (1983).
3. D. Aviv and E. Galun, *Planta Med.*, **33**, 70 (1978).
4. T. Suga and T. Hirata, *Phytochemistry*, **29**, 2393 (1990).
5. T. Hirata, H. Hamada, T. Aoki, and T. Suga, *Phytochemistry*, **21**, 2209 (1982).
6. T. Suga, T. Hirata, H. Hamada, and S. Murakami, *Phytochemistry*, **27**, 1041 (1988).
7. P. Brodelius, B. Deus, K. Mosbach, and M.H. Zenk, *FEBS Lett.*, **103**, 93 (1979).
8. Y.X. Tang and T. Suga, *Phytochemistry*, **31**, 2599 (1992).
9. T. Suga, S. Izumi, and T.Hirata, *Chem. Lett.*, 2053 (1986).
10. R. Croteau, *Chem. Rev.*, **87**, 929 (1987).
11. T. Murashige and F. Skoog, *Physiol. Plant.*, **15**, 473 (1962).

Received 30 September 1992